BAOMS Research Grant –

Interim Report - March 2019

Mr Karl F. B. Payne

Date awarded - March 2018. Grant received – October 2018 - £9,813

Project title:

Circulating tumour DNA as a liquid biopsy and biomarker in head and neck squamous cell carcinoma (HNSCC)

Supervisors: Professor Hisham Mehanna and Mr Paul Nankivell

Institution – University of Birmingham

Background:

Despite advances in treatment, survival in HNSCC remains static and the incidence of recurrence/metastasis (R/M) is high. Tumour biopsies can rarely be taken from such R/M patients and treatment selection is therefore empirical, with consequent low rates of response. Furthermore, because HNSCC has significantly high intra-tumour genomic heterogeneity, any such samples cannot capture the entire mutational landscape of the tumour - thereby limiting their usefulness and leaving the clinician blind as to the emergence of clonal mutations driving resistance. A liquid biopsy utilises circulating fragments of tumour DNA (ctDNA) or tumour cells (CTCs) to assess tumour specific genomic alterations. This novel technique promises to deliver a non-invasive method to detect tumour specific heterogenous genomic alterations in a dynamic fashion and guide targeted therapy.

Research question:

Can ctDNA be used as a liquid biopsy in HNSCC to assess tumour heterogeneity and a biomarker to detect tumour recurrence?

Experimental design:

The initial epxerimental design of this project was to perform tumour tissue whole exome and then targeted sequencing of ctDNA blood samples from 9 HNSCC

patients. However, initial extraction from blood samples revealed low quantities of ctDNA. While this is expected from this assay we have concern regarding the quality of sequencing data that will be obtained, given the high cost to run such tests. To solve this problem we are investigating various amplification or low input library preperation methods to negate the low quantiity of ctDNA and still obtain high quality data.

Therefore, to progress the project we made the decision to focus on methylation specific epigenetic alterations of the ctDNA. We were more confident that the low quantity of ctDNA would still yield meaningful data on a new methylation array.

Results:

- Tumour tissue and blood samples were obtained from 6 HNSCC patients. ctDNA was extracted and run on an Illumina Human Methylation 450K Bead Chip.
- Results from this method have been very promising. Despite running ctDNA volumes up to 10x lower than recommended ranges we obtained adeqaute beadchip CpG methylation site coverage.
- Initial bioinformatics analysis sought to compare methylation patterns in tissue DNA and ctDNA, to validate this technique to detect tumour specific epigenetic alterations. This has revealed high concordance between tissue and ctDNA samples. Further in-depth data analysis to evaluate gene locus specific alterations is ongoing.

Planned presentation of findings:

- An oral presentation abstract has been submitted and accepted at the BAOMS 2019 scientific meeting - ***High-throughput methylation profiling of cell-free plasma DNA in head and neck cancer: a pilot study***
- Mauscript in preperation for submission to the *British Journal of Oral and Maxillofacial Surgery*, as per agreement.

Future work:

- The protocol will continue to be optimised to enable targeted sequencing of ctDNA, as planned in the original proposal. This work will aim to be completed within the one-year research grant as proposed.
- The remaining funds from the BAOMS research grant will be used to complete the targeted sequencing, supplemented by a Wellcome ISSF grant held by Mr Paul Nankivell.